Heat stress and genotype affect the glutenin particles of the glutenin macropolymer-gel fraction

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Abstract

Genetic and environmental factors that affect the formation of glutenin macropolymer (GMP) particles were investigated by growing Lance $5+10$, Lance $2+12$, Warigal $5+10$ and Warigal $2+12$ lines under widely differing temperature regimes in greenhouse conditions. The GMP characteristics and mixing properties of flour extracted from mature wheat kernels were systematically studied. The amount of GMP is more sensitive to growing conditions than protein quantity. With the exception of severe heat stress conditions, $5+10$ varieties produce larger glutenin particles than $2+12$ varieties. The HMW/LMW ratio of GMP is lowered by heat stress, but glutenin particles become larger. In heat stress effects on dough mixing properties, glutenin particle size is more relevant than GMP quantity for dough development time; and GMP quantity is more relevant for band-width at peak resistance. A hyperaggregation model is used to explain how heat stress controls glutenin particle formation.
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1. Introduction

Differences in wheat quality are known to be affected by both genetic and environmental factors. Growing conditions can affect the quality of any variety. The amount of glutenin macropolymer (GMP) in flour parallels differences in wheat flour bread-making quality (Graveland et al., 1982; Graveland, 1984; Weegels et al., 1996, 1997). GMP consists of HMW and LMW subunits. HMWGS and LMWGS present in glutenins are linked by disulphide bonds. Recently it was shown that GMP consists of spherical glutenin particles (Don et al., 2003a). Size measurements revealed that glutenin-particles can vary in average size. Flour from '$2+12$'variety Estica was shown to have, on average, less

GMP and smaller glutenin particles than flour from the '$5+10$'variety Baldus. Clearly, genetic background affects GMP quantity, GMP particle size and, consequently, flour quality. Glutenins are formed during wheat kernel development (Carceller and Aussenac, 2001). Therefore, growing conditions are also expected to affect the formation of GMP. To study this in more detail in combination with effects of genetic background, near isogenic lines: Lance C ('$5+10$'), Lance A ('$2+12$'), Warigal A ('$5+10$') and Warigal B ('$2+12$'), were grown under six different greenhouse conditions (Don et al., 2003c). These six growing conditions were used to mimic various heat stress conditions. The GMP characteristics of the Warigal and Lance lines were studied to better understand the effects of genetic background and heat stress on glutenin particle formation and mixing properties.

Abbreviations: BWPR, band-width at peak resistance; CSLM, confocal scanning laser microscopy; DAA, days after anthesis; DDT, dough development time; E, surface area; GMP, glutenin macropolymer; HMW-GS, high molecular weight glutenin subunits; IPP, insoluble polymeric protein; LMWGS, low molecular weight glutenin subunits; UPP, unextractable polymeric protein..

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2. Experimental

2.1. Wheat materials

Four near-isogenic wheat lines were used, Lance C (HMWGS 2*, $17+18$, $5+10$) + Lance A (HMWGS

2*, 17+18, 2+12) and Warigal A (null, 7+8, 5+10) + Warigal B (null, 7+8, 2+12). Wheat was grown in pots under controlled conditions in greenhouse facilities at Kansas State University. Each of the pots in the greenhouse was automatically watered three times a day using the local water supply. In the growth chambers, where the heat treatments were applied, the pots were kept under close surveillance to maintain the moisture supply and were watered at least once a day. The aim was to study the effects of heat stress free from conditions of water stress. The wheat lines were grown to maturity, using various temperature regimes—t °C day/t °C night—to simulate six different stress levels (Table 1). Protein contents of powdered kernel materials were determined by nitrogen combustion (LECO) on 200 mg samples and averages of duplicates calculated.

2.2. Wheat milling on Quadrumat JR

Wheat kernels ($\sim$30 g) were milled on a Quadrumat JR (Brabender). Kernel moisture was adjusted to 14–17% depending on the hardness of the grain, bran was separated from endosperm flour by sieving over a 150 μm sieve. Due to effects of growing conditions on kernel filling, extraction rates varied between 50 and 70%, noting that samples grown under condition #5 (Table 1) had a typically low flour extraction of about 50%. These kernels had a shrivelled appearance. For samples from growing conditions #1, #2, and #3 extraction was ca 70%, for #4 and #6 it was 60%.

2.3. Dough mixing

Mixing properties were determined with a National 2 g Mixograph. Water was added according to AACC method 54–40 including addition of 2% salt (NaCl). The dough development time (DDT), Band Width at Peak Resistance (BWPR) and surface area (E), that parallels average energy-input for optimal development, were recorded.

2.4. Characterization of GMP-gel and GMP dispersions

GMP was isolated by dispersing flour in 1.5% SDS followed by ultracentrifugation as described earlier by Graveland et al. (1982) The GMP gel-layer rigidity was measured with a Bohlin VOR (Bohlin, Sweden) as described by Wang (2003). Particle properties were determined viscometrically and with a Coulter Laser LS32 particle sizer as described by Don et al. (2003b). From the Coulter Laser pattern, derived diameters can be calculated, such as $D_{3,2}$ (weighted average surface area of the particles) and $D_{4,3}$ (weighted average volume).

Particles were also observed with confocal scanning laser microscopy (CSLM) as described by Don et al. (2003b).

2.5. Protein content of flour and GMP samples

Protein content of flour, GMP, GMP dispersions and SDS extractable proteins were measured using UV absorption calibrated with a set of Kjeldahl protein values for GMP.

2.6. RP-HPLC analysis of flour glutenins and GMP

A Waters HPLC with C8 column was used according to Lookhart et al. (2003), with some adaptations (Weegels et al., 1996) for the 50% isopropanol + DTT extracted glutenins from flour and glutenins present in the GMP-gel fraction.

3. Results

3.1. Effect of growing conditions on GMP protein in flour

The Lance and Warigal wheat samples were grown under the conditions shown in Table 1. Flour was extracted from the grains as described in Section 2. The flour protein and GMP content varied with growing conditions for the Lance and Warigal lines. The severity of the heat stress conditions increased from treatment #1 to #5 (Table 1). With treatment #6, the period of heat stress (40 °C day time) started later than with treatment 5 (25 DAA instead of 16). In general, total flour protein content ranged between 12 and 17%. Comparison of flour protein content between non-stressed and slightly stressed (#1 and #2) conditions, showed a systematic difference, with the 5+10 lines having a higher flour protein content than the 2+12 lines. Since growing

Table 1

Greenhouse growing conditions #1–#6, day/night temperature used with Lance and Warigal wheat lines

#1. 20°C/16°C -- 40 days → mature
#2. 20°C/16°C -----→16DAA --72h at 30°C/18°C -→ 20°C/16°C -----37 - 38 days → mature
#3. 20°C/16°C -----→16DAA --72h at 35°C/20°C-→20°C/16°C-----35 - 36 days → mature
#4. 20°C/16°C -----→16 DAA -- 35°C/20°C--------------------30 - 31 days → mature
#5. 20°C/16°C -----→16 DAA -- 40°C/25°C------------25 days → mature
#6. 20°C/16°C -----→25 DAA -- 40°C/25°C--------------28 days → mature

Table 2
The % of protein in flour and the % of GMP protein in flour protein

Growing condition	Lance 2+12		Lance 5+10		Warigal 2+12		Warigal 5+10	
	Protein % in flour	%GMP in protein	Protein% in flour	%GMP in protein	Protein% in flour	%GMP in protein	Protein% in flour	%GMP in protein
#1	12.7±0.4	20.2±1.7	15.4±0.7	34.6±2.4	13.5±0.6	21.1±1.8	14.7±0.6	27.2±2.0
#2	13.2±0.5	18.6±1.5	16.4±0.8	34.7±2.5	14.4±0.7	22.0±1.8	14.2±0.7	28.6±1.9
#3	11.9±0.6	19.1±1.8	16.7±0.8	29.3±2.1	14.2±0.7	23.3±1.9	14.1±0.6	30.0±2.2
#4	13.2±0.5	15.1±1.3	15.4±0.6	23.3±1.8	14.5±0.6	16.0±1.4	14.4±0.6	22.9±1.7
#5	11.7±0.5	9.0±0.8	14.2±0.7	19.5±1.6	13.6±0.6	9.7±1.0	13.3±0.5	13.3±0.9
#6	12.7±0.6	16.4±1.5	14.5±0.7	30.2±2.2	12.2±0.6	15.6±1.4	13.6±0.5	20.2±1.5

conditions are the same, this can only be due to genotypic differences.

From Table 2 no systematic effect on % protein can be observed for effects of stress, perhaps with the exception of #5. Heat stress can affect starch biosynthesis more than protein biosynthesis (Blumenthal et al., 1995; DuPont and Altenbach, 2003; Spiertz et al., unpublished). Also the results show that with increasing heat stress protein content per kernel decreases and protein concentration increases (Naeem et al., 2002), illustrating again the different effects of stress on protein and starch biosynthesis. This effect is less clear in flour extracted from these kernels, perhaps due to the different rates of extraction that were achieved. Only in condition #5 was the protein content decreased marginally.

In the context of this paper we focused on the effects of stress on GMP, a protein fraction that can directly be related to quality.

The effect of stress conditions on the content of GMP is already apparent at less severe stress conditions (from #3 to #6, Table 2). Heat stress causes a reduction in GMP to a greater extent than for protein (up to 40–50%). Differences are also noted between near isogenic lines. The 5+10 lines typically have a higher GMP content than the 2+12 lines as observed by Payne et al. (1987). Interestingly, Lance 5+10 seems to be more resistant to heat stress than the other three lines. These results confirm the positive effect of HMW-GS 5+10 on both protein and GMP formation. The variations observed underline that phenotypic as well as genotypic effects are important. Whereas genotype affects protein and GMP levels under non-stressed conditions, stress conditions seem dominant with respect to GMP content.

In growing condition #5 the application of heat stress from 16 DAA onwards negatively affects GMP formation. Again, this sharp decrease in GMP cannot be explained by a parallel decrease in flour protein or kernel protein content.

This effect of heat stress was not expected since Carceller and Aussenac (2001) showed that heating is essential for the formation of GMP and that they could induce GMP formation by heating immature endosperm (without GMP). Comparison of conditions #5 and #6, also demonstrates that the timing of the application of heat stress is critical. In condition #6, heat is increased at a later stage (from 25 DAA onwards) and significantly more GMP is formed (e.g. with Lance 5+10: 30.2% vs 19.5%). This indicates a negative effect of heat in the early stages of glutenin synthesis.

3.2. Effect of growing conditions on GMP-gel stiffness and GMP wet weight

GMP and its rheological properties are reported to be good quality predictors (Graveland et al., 1982; Graveland, 1984; Kelfkens and Lichtendonk, 2000; Pritchard, 1993; Weegels et al., 1996, 1997). Therefore, we investigated how the rheological properties of GMP were affected by growth conditions. Figs.1a–d show how GMP stiffness (G') and GMP wet weight vary with the different growing conditions (#1–#6 in Table 1). As expected, GMP wet weight (Fig. 1a–b) follows the trend for the %GMP proteins (Table 2). The amount of GMP gel (Fig. 1 a–b) and the %GMP protein (Table 2) are highly correlated ($R^2 = 0.94$) for the whole set of lines and heat stress conditions ($n = 24$).

GMP gel stiffness is related to both the concentration of glutenin proteins and the average voluminosity of the glutenin particles in the GMP gel (Don et al., 2003a). Gel stiffness initially (#1–3) parallels the amount of GMP and is constant. Also, GMP values for 5+10 lines are considerably higher than for 2+12 lines. However, striking differences in GMP quantity and GMP stiffness are observed for growing regimes #4, #5 and #6. With treatments #4 and #6 a decrease in GMP is observed, but GMP stiffness does not follow this trend. With treatment #5 a more dramatic decrease in the amount of GMP is observed, but in contrast, GMP stiffness increases, especially for the 2+12 lines, where stiffness values are higher than for the 5+10 lines (Fig. 1c–d).

Treatment #6, where heat stress treatment commenced later (from 25 DAA onwards). GMP stiffness is more comparable to the less severe treatments.

Heat stress between 16 DAA and 25 DAA markedly affected the rheological properties of the glutenin fraction GMP, as shown by the increase in G' values with heat stress. We have shown (Don et al., 2003b) that G' can be described as a function of glutenin particle voluminosity and protein concentration. Protein concentrations are not affected or even
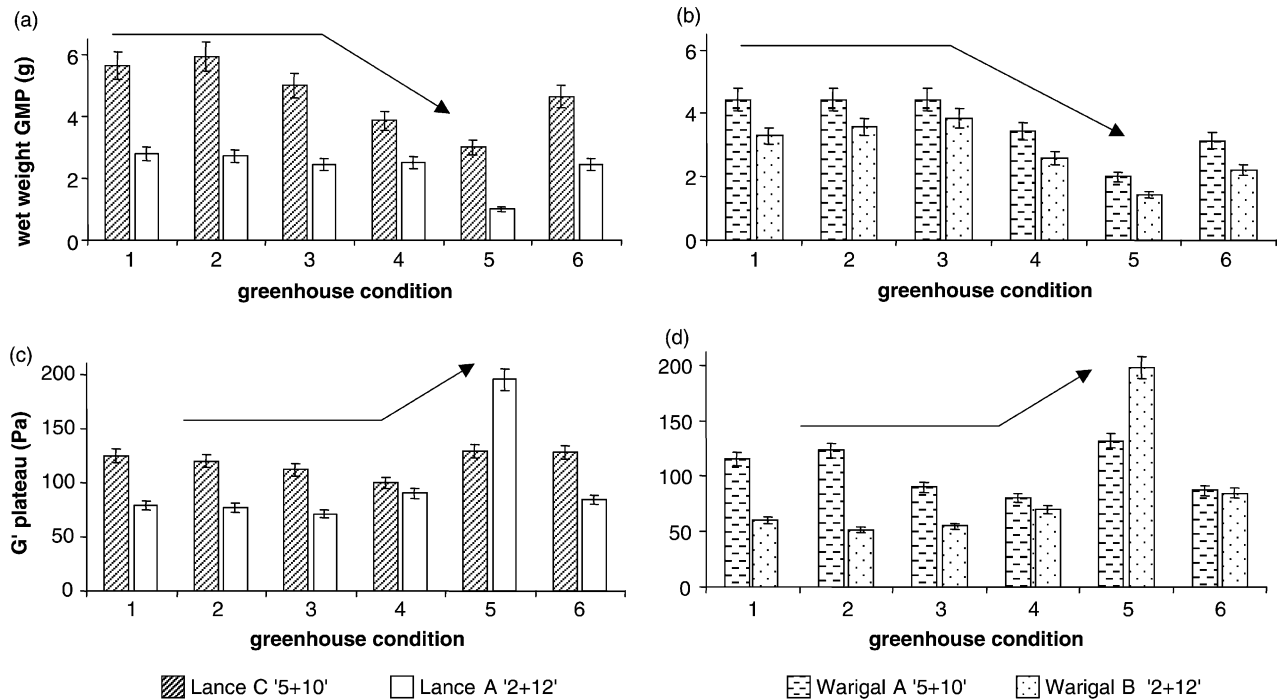
Fig. 1. (a–d) The effect of growing conditions #1–#6 on GMP wet weight (g) and GMP-gel stiffness (G') for wheat lines Lance $5+10$, Lance $2+12$, Warigal $5+10$ and Warigal $2+12$.

decreased. Therefore, the increase in G' could be an indication that heat stress leads to the formation of larger particles. This seems to hold especially for the $2+12$ lines in treatment #5, where G' values exceed the values of the $5+10$ lines. In parallel to the strong increase in G' of the $2+12$ varieties, the phase angle δ decreases from $11°$ for condition #1 to $9°$ for condition #5. This decrease in δ confirms that the GMP-gel becomes more elastic with heat stress, indicative of a different internal structure of the glutenin particles.

To learn more about the specific nature of the glutenin particles present in GMP (Don et al., 2003a,b), we therefore, studied how growing conditions affected GMP-particle size in more detail.

3.3. Glutenin particle size distributions

The glutenin particles in GMP were measured by Coulter Laser analysis. Figs. 2 and 3 show the different size distributions for the various wheat lines and treatments. Particle sizes ranged between 1 and ca 300 μm. From the size distributions a characteristic parameter, the $D_{3.2}$ (the volume to surface ratio) can be calculated. In general, $D_{3.2}$ values increase with increasing heat stress. The distribution observed with treatment #5, stands out and is characterised by a fraction with very large particles >100 μm. Although most prominent with the $2+12$ lines, this feature is observed with all wheat lines and seems characteristic for this treatment. This feature seems to be caused by the early application of heat and/or longer exposure, since it is not found with treatment #6.

3.3.1. Relation between G' and glutenin particle size

Fig. 4a confirms the earlier results of Don et al. (2003b) that the GMP-particle voluminosity, determined from GMP dispersion viscosity, correlates with G' indicating that protein concentration is less important here. As expected, the $D_{3.2}$ data from the Coulter laser measurements and G' also show a positive correlation (Fig. 4b). Fig. 4a reveals a number of interesting features regarding the effect of both genotype and heat stress. First, genotype seems to control the baseline level of size, with the $5+10$ lines (filled symbols) giving larger particles than the $2+12$ lines (open symbols). However, systematic differences also exist between Warigal and Lance. Lance $2+12$ gives more voluminous particles than Warigal $2+12$, Lance $5+10$ gives larger particles than Warigal $5+10$. These differences could be related to the differences in HMWGS composition.

The average glutenin particle sizes resulting from growing conditions were of the following order: #1 < #2 < #3 < #4 ≤ #6 ≪ #5. In general, heat treatment leads to the formation of larger particles, with $5+10$ lines giving larger particles than $2+12$ lines. The results for the most stressful treatment (#5) are shown in Fig. 4a and b. The combination of treatment #5 and $2+12$ resulted in extremely large particles and is exceptional (encircled data points). Here, the $2+12$ lines form larger particles than the $5+10$ lines. For clarity we have summarized the particle size data of all treatments in Table 3. This shows that fewer, smaller particles ($D<10$ μ) are formed with increasing stress and that area% of $D>100$ μ increases with stress. $D_{3.2}$ (weighted average surface area of the particles) is useful
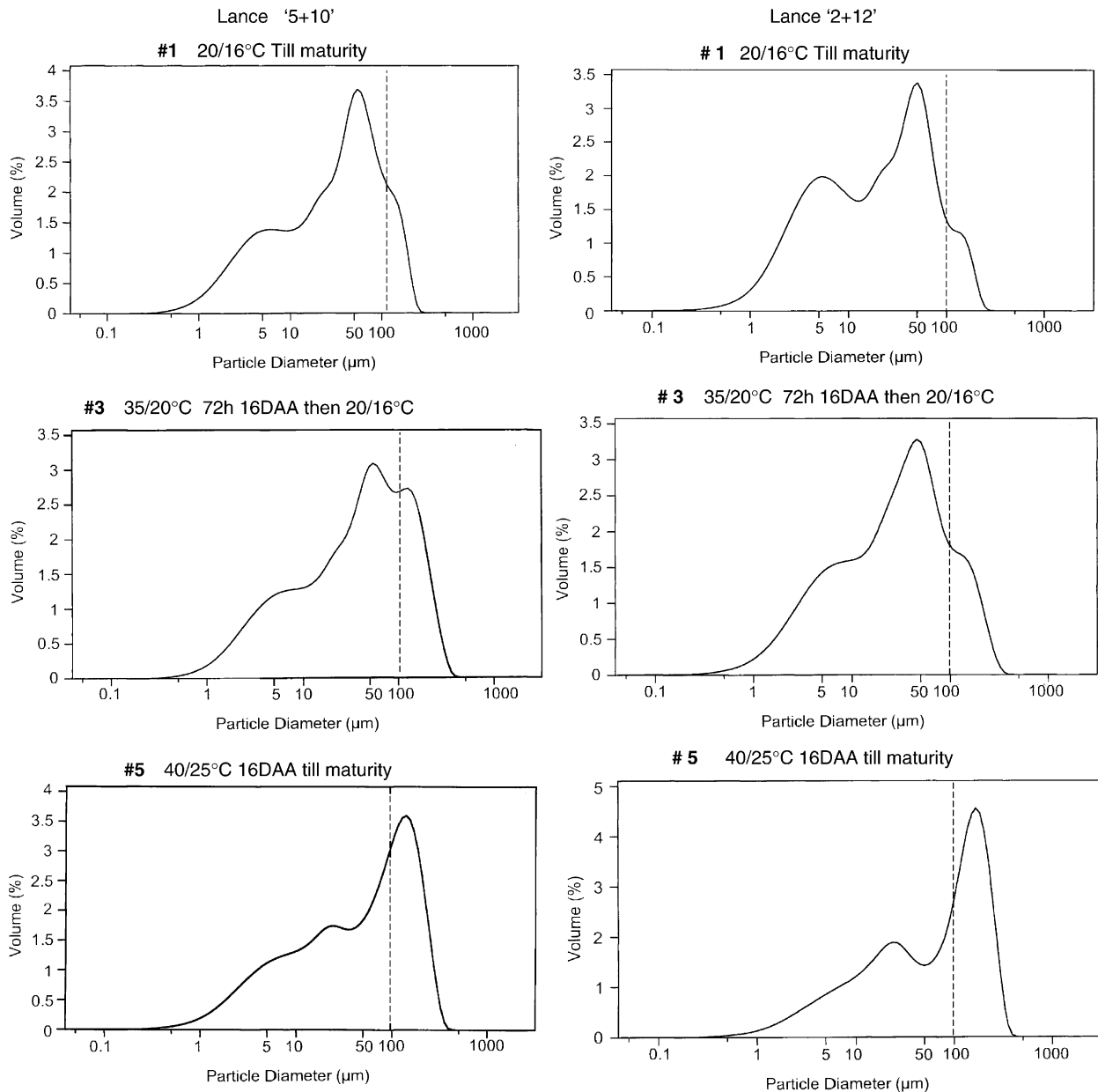
Fig. 2. The effect of growing conditions #1, #3 and #5 on the glutenin particle size distribution of wheat lines Lance 5 + 10 and Lance '2 + 12'.

for predicting particle gel structural properties (Fig. 4). The parameter $D_{4.3}$ (weighted average volume) better accounts for the larger particles. Table 3 shows that $D_{4.3}$ responds sensitively to stress effects. $D_{3.2}$ and $D_{4.3}$ correlate ($R^2 = 0.77$). Also the viscometrically determined voluminosity parameter, η, correlates with $D_{3.2}$ and $D_{4.3}$ ($R^2 = 0.78$ and 0.62, respectively).

3.3.2. CSLM observations of glutenin particles

To confirm the trends indicated with Coulter and viscosity measurements, we also observed the glutenin particles using CSLM. Fig. 5a–d shows the most contrasting samples (treatment #1 vs #5 for Lance). In particular with the $2 + 12$ variety some very large and more irregularly

shaped particles are seen (arrows in Fig. 5d), that seem to consist of fused smaller particles. Again, this is only observed with treatment #5 and $2 + 12$ lines. This confirms the very large particle diameters observed with Coulter laser.

3.4. HMWGS-LMWGS ratio and effect on glutenin-particle size

Heat stress could have an effect not only on size, but also on the composition of the glutenin particles. Therefore, the HMWGS and LMWGS composition of both flour and GMP, for all samples, was determined. RP-HPLC profiles for treatments #1 and #5 are compared in Fig. 6. Qualitatively,
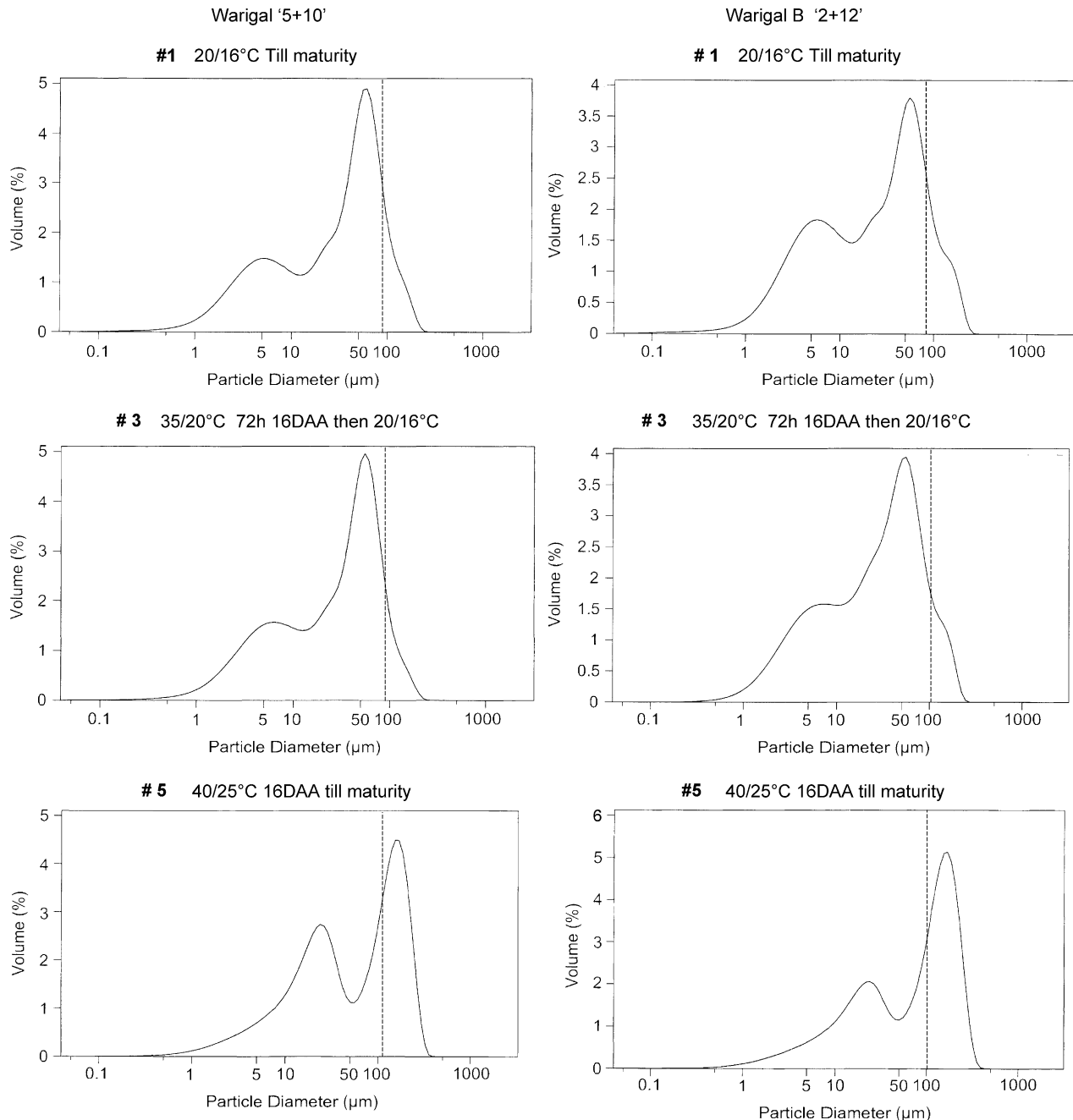
Fig. 3. The effect of growing conditions #1, #3 and #5 on the glutenin particle size distribution of wheat line Warigal '5 + 10' and Warigal' 2 + 12'.

the patterns are the same so that heat treatment did not result in different levels of expression of specific HMW or LMW glutenin subunits. Similarly, DuPont and Altenbach (2003) did not find any evidence that expression of *specific* glutenin subunits changed with heat stress. However, they did find that heat stress led to a decrease in the glutenin / gliadin ratio.

Our HPLC data showed a change in the *relative amount* of HMWGS over LMWGS (HMW/LMW ratio). The HMW/LMW ratios for both in flour and GMP were calculated and, in general, they are the same. This contrasts

with the results of Don et al. (2003) where the ratio in GMP was 1.6 times the ratio in flour. We cannot provide a conclusive explanation for this difference. However, we note that the ratio of 1.6 refers to a group of European wheat varieties. The US lines used in this study, and other US lines analysed in our laboratory, have a significantly higher HMW/LMW ratio in flour (US vs EU: HMW/LMW 0.9–1.4 (N=7) vs 0.2–0.7 (N=10)). This may explain why the GMP HMW/LMW ratio for the US samples used in these experiments is similar to the HMW/LMW ratio in the respective flours. For EU samples HMW/LMW in flour is
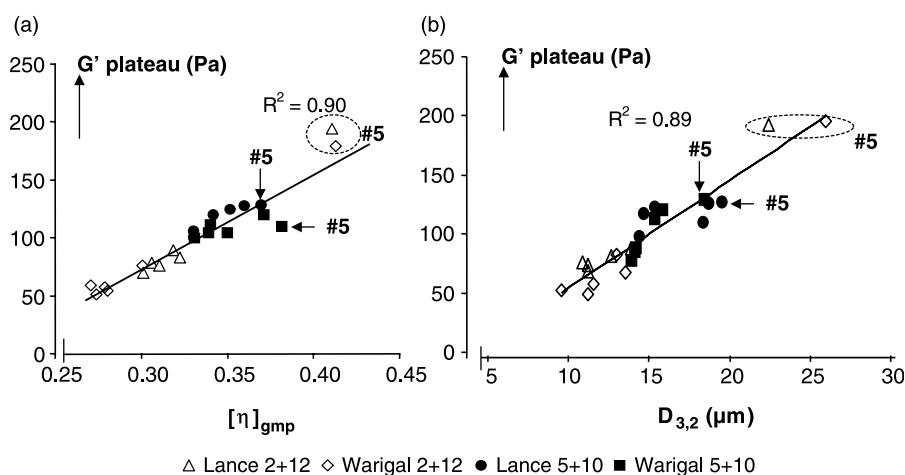
Fig. 4. (a) Relation between G' plateau values of GMP-gel and the effective glutenin particle voluminosity $[\eta]$ for wheat lines Warigal and Lance. (b) Relation between G' plateau values of GMP-gel and glutenin particle volume surface ratio $D3.2$.

lower, and perhaps also there are bigger differences in GMP HMW/LMW and flour HMW/LMW (ca 1 vs 1.6).

With increasing heat treatment, the relative amount of LMW increases, leading to a lower HMW/LMW ratio (#1 vs #5 = 1.3 vs 1.0). We could not detect differences between Warigal and Lance or between 5 + 10 and 2 + 12 lines. We therefore, consider this decrease in ratio to be an effect of the heat stress. Interestingly, with treatment #6 where heat stress is applied at a later stage and consequently for a shorter duration, HMW/LMW ratio is ca 1.2. This suggests that heat stress, applied as early as 16 DAA, affects HMW and LMW glutenin synthesis differently, lowering HMW/LMW ratio.

Considering all treatments, a relationship is observed between $D_{3.2}$ and the HMW/LMW ratio (Fig. 7a–b), with decreasing HMW/LMW, the $D_{3.2}$ increases. Nevertheless it cannot be excluded that both an increased size and a decreased ratio are the result of heat stress. The effect of the combination of treatment #5 with 2 + 12 again, is unexpected. In unpublished experiments we have shown that 2 + 12 varieties have smaller glutenin particles than 5 + 10 varieties. However, the present experiments gave the opposite result: with treatment #5 the glutenin particle size of 2 + 12 varieties is larger than that of 5 + 10 varieties. Furthermore, the HMW/LMW ratio is significantly changed by heat stress (<1) and the $D_{3.2}$ is sharply increased ($D_{3.2} > 20$). Glutenin particles consist of both HMWGS and LMWGS and we have shown that HMWGS are central to the formation of glutenin particles (Don et al., 2003d). LMWGS alone cannot form particles although they can become part of a particle with HMWGS. It is likely that a variation in the ratio between HMWGS and LMWGS will affect particle size. Don et al. (2003d) have shown that under unstressed growing conditions, increasing HMW/LMW ratios result in increasing glutenin particle sizes. Nevertheless, we believe that the enormous increase in size observed with treatment #5 is the result of a different process and must be considered an abnormal effect of the very high temperature stress.

3.5. Micro-scale mixing experiments

Mixograph tests are an important tool in assessing technological quality differences on a small scale. Dough development time (DDT) is related to glutenin content and

Table 3
Summary of the Coulter Laser glutenin particle size data for all 24 samples

|	$D < 10$ (area%)	$10 < D < 100$ (area%)	$D > 100$ (area%)	$D_{3.2}$ (μm)	$D_{4.3}$ (μm)
Lance 2 + 12					
1	36.4	55.1	8.6	10.8	42.8
2	29.1	59.7	11.2	11.0	44.5
3	26.7	58.3	15.0	11.1	46.8
4	29.0	50.2	20.9	12.0	55.1
5	16.2	41.3	42.5	22.3	94.7
6	29.7	52.3	18.0	12.2	60.5
Lance 5 + 10					
1	26.4	59.4	14.2	14.4	47.1
2	26.8	61.6	11.7	14.7	46.2
3	22.8	54.3	22.9	15.1	63.9
4	20.9	51.0	28.1	18.0	67.9
5	22.1	45.4	32.5	19.8	77.0
6	20.3	55.0	24.8	18.1	65.5
Warigal 5 + 10					
1	27.1	63.9	9.0	13.8	43.8
2	28.2	64.6	7.2	13.9	42.3
3	27.1	66.7	6.2	14.1	39.6
4	26.5	57.6	15.9	15.0	50.2
5	14.2	46.8	39.0	18.1	86.1
6	27.7	56.7	15.6	15.6	50.3
Warigal 2 + 12					
1	32.8	58.2	9.0	9.0	41.1
2	28.1	63.8	8.1	11.2	41.3
3	27.9	62.8	9.4	11.8	42.0
4	27.5	54.5	18.0	13.1	52.1
5	15.2	40.4	44.4	25.1	96.4
6	29.7	46.8	23.5	14.0	59.3

T #1   T # 5
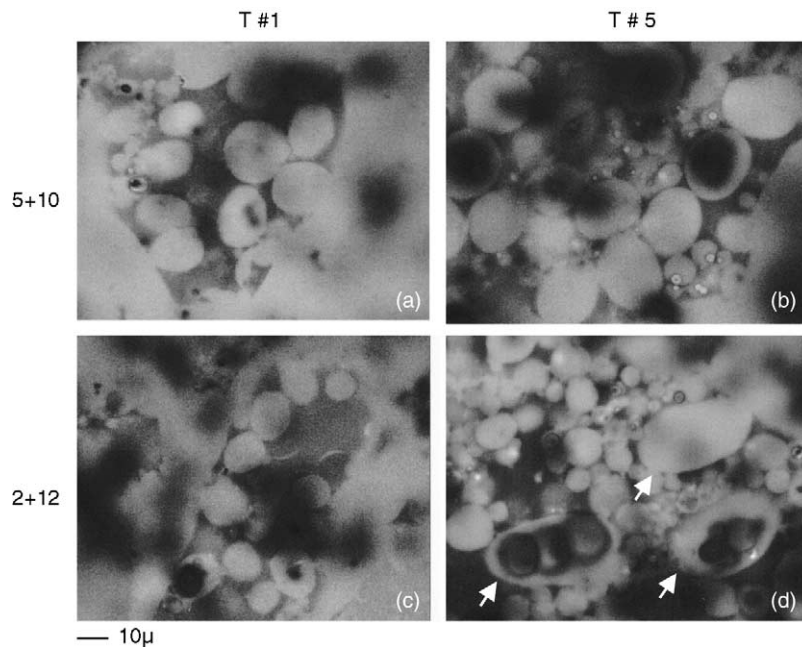
Fig. 5. (a–d) Examples of CSLM pictures that show how glutenin particle sizes are strongly affected by both HMWGS composition (2+12 vs. 5+10) and growing conditions (#1 vs. #5).

recognized as an indicator of dough strength. The bandwidth at peak resistance (BWPR) has been correlated with extensogram Rmax (Uthayakumaran et al., 1999). The heat stress conditions induced a unique variation in GMP quantity and glutenin particle size. Since glutenin-particle size increases with heat stress, DDT is also expected to increase (Don et al., 2003b). On the other hand, DDT is also reported to increase with the amount of GMP (Graveland et al., 1984; Weegels et al., 1996, 1997), so that DDT would decrease with heat stress. As expected, the results with the 2 g mixograph revealed a wide variation in DDT values.

In Fig. 8a DDT is plotted against GMP wet weight. Taking all samples together there is no correlation. However, if we exclude samples from treatment #5, a weak relation ($R^2 = 0.5$) is seen. With decreasing GMP, DDT also decreases. However, if we plot DDT against glutenin particle size (Fig. 8b), a better correlation is observed, even when all samples are included ($R^2 = 0.78$). Apparently, glutenin particle size is more important than GMP wet weight for DDT. This is in agreement with Don et al. (2003a,b,c,d) who proposed a mechanism in which optimal dough mixing is governed by glutenin
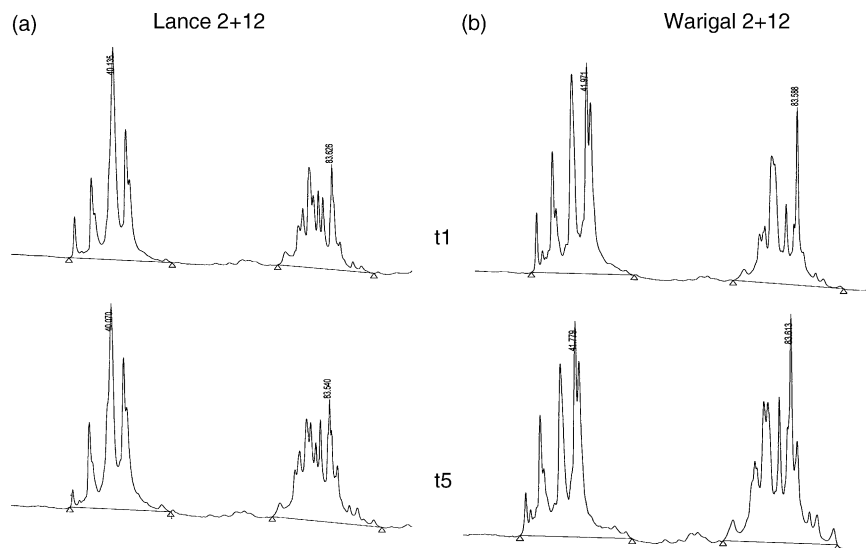

Fig. 6. (a–b) Examples of RP-HPLC traces that show how the two most differing growing conditions (#1 vs. #5) affect the HMW/LMW of lines Lance 2+12 and Warigal 2+12.
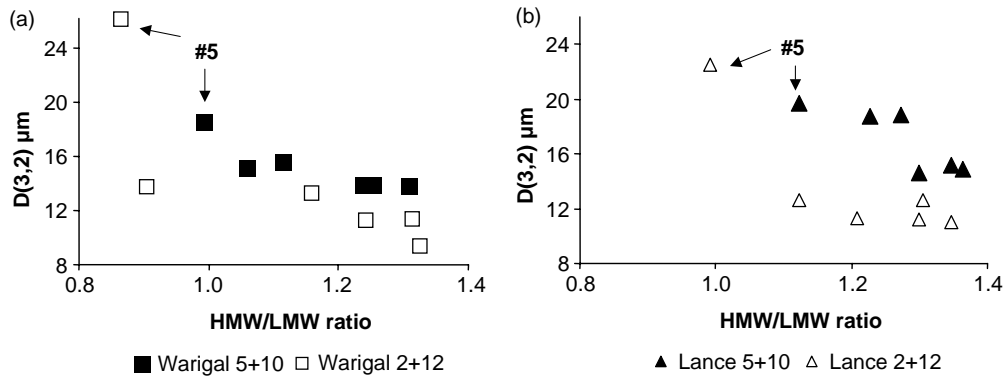
Fig. 7. (a–b) The effect of growing conditions #1–#6 on GMP HMW/LMW ratio and how this affects the glutenin particle $D_{3.2}$ of wheat lines Lance 5+10, Lance 2+12, Warigal 5+10 and Warigal 2+12.

particle disruption. Nevertheless, GMP wet weight is still relevant for dough properties. Fig. 9a shows a correlation of $R^2 = 0.83$ between BWPR and GMP wet weight. Glutenin particle size does not correlate with BWPR (Fig. 9b).

4. Discussion

4.1. Heat stress effects on dough mixing properties

It is generally accepted that wheat quality is the result of genotype x growing condition. Thus there is a large variation in wheat quality, not only between varieties, but also between different batches/growing locations of the same variety. Although flour millers aim to produce a uniform flour product, the baker is still faced with variations, especially with respect to dough mixing requirements. For this reason there is a strong interest in understanding the nature of these variations. With un-stressed wheat samples there is, in general, a relationship between gluten, glutenin or

GMP- content and dough mixing requirements (Bekkers et al., 2000; Gupta et al., 1992; Lafiandra and MacRitchie, 1997; Pritchard, 1993; Weegels et al., 1996, 1997). Using a unique set of heat stressed samples important details of this relationship were uncovered. It is clear that not only the amount of GMP, but also the size of the glutenin particles contained in this fraction are important. This information could help manage heat stress effects, if we understood how heat stress effects glutenin particle formation. Heat stress influences both glutenin biosynthesis and glutenin particle formation. In general, we observed that with increasing heat stress (treatments #4–6), less GMP is formed, but that this fraction contains larger glutenin particles. The decrease in polymeric glutenins like GMP is confirmed by measurement of %UPP (Unextractable Polymeric Protein) (Naeem et al., 2002) or %IPP (Insoluble Polymeric Protein) (Ciaffi et al., 1996). We agree with Ciaffi et al. (1996) who suggested that that this was due to a decrease in HMW-GS synthesis. The new observation that larger particles are formed helps explain how stress affects dough mixing time. Carceller and Aussenac (2001) suggested that dehydration and heat induce
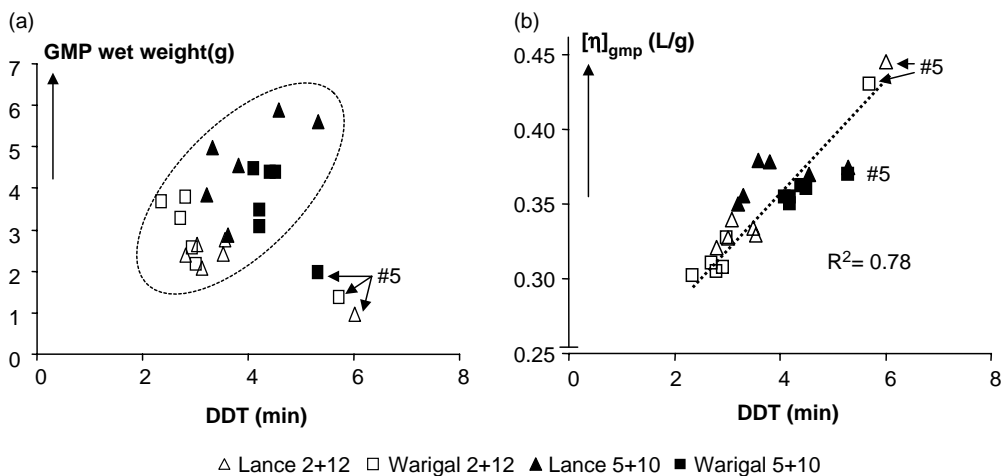

Fig. 8. (a) Relationship between DDT and GMP wet weight for Lance 5+10, Lance 2+12, Warigal 5+10 and Warigal 2+12 grown under conditions #1–#6. (b) The relationship between DDT and glutenin particle voluminosity [η] for Lance 5+10, Lance 2+12, Warigal 5+10 and Warigal 2+12 grown under conditions #1–#6.

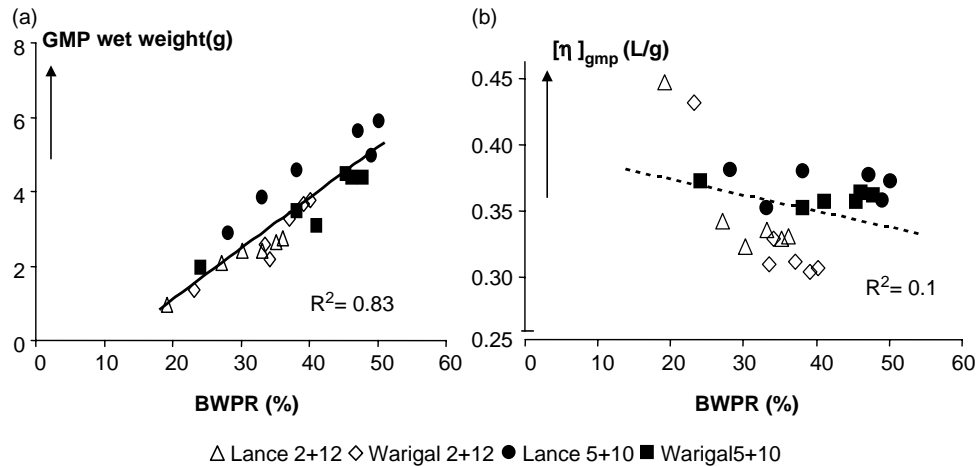△ Lance 2+12 ◇ Warigal 2+12 ● Lance 5+10 ■ Warigal 5+10

Fig. 9. (a) The relationship between BWPR and GMP wet weight for Lance $5+10$, Lance $2+12$, Warigal $5+10$ and Warigal $2+12$ grown under conditions #1–#6. (b) The relationship between BWPR and glutenin particle voluminosity $[\eta]$ for Lance $5+10$, Lance $2+12$, Warigal $5+10$ and Warigal $2+12$ grown under conditions #1–#6.

a more rapid and increased disulphide bond formation and glutenin aggregate formation. However, aggregation phenomena are also related to the amount of protein, and we expect that changes in the HMW/LMW ratio also play a role. These considerations led us to the following hypothesis to explain the effects of heat stress on glutenin particle formation.

4.2. Hypothetical formation of glutenin particles

If we apply the hyper-aggregation model (Carceller and Aussenac, 2001; Hamer and van Vliet, 2000) for the formation of a glutenin particle, we can distinguish three steps:

(1) Biosynthesis of glutenin subunits (HMW plus LMW).
(2) Polymerisation and formation of larger oligomer clusters (Carceller and Aussenac, 2001) and observable protein storage vacuoles at ~ 14–17 DAA (Bechtel and Wilson, 1997).
(3) Further assembly into larger insoluble glutenin particles (Carceller and Aussenac, 2001), and also fusion of protein particles sometimes observable during maturation (Bechtel and Wilson, 1997).

The hypothetical assembly process is depicted in Fig. 10. We suggest that assembly of the glutenins during steps 2 and 3 is important in controlling the final size of the glutenin-particles. When heat stress starts at 16 DAA as in #5, the higher temperature slows the biosynthesis of glutenin (step 1) as reported also by DuPont and Altenbach (2003). This explains the observed overall decrease in GMP. On the other hand, higher temperatures can cause the hyper-aggregation in steps 2 and 3 to progress faster (Carceller and Aussenac, 2001). This could explain why from a smaller amount of glutenin, even larger particles are formed.

In general, the $5+10$ varieties have more GMP than $2+12$ varieties, irrespective of the growing regime.

This could result from a higher content of glutenins as reported by DuPont and Altenbach (2003) which would affect all steps of the hyperaggregation model.

We also note that with the exception of treatment #5, $5+10$ varieties produce bigger particles than $2+12$ irrespective of GMP content. This could be due to the specific polymerisation properties of $5+10$ lines (Lafiandra et al., 1993; Popineau et al., 1994). Also, $5+10$ lines typically have a higher HMW/LMW ratio than $2+12$ lines as observed earlier (Don et al., 2003b). Apparently, these features enable $5+10$ lines to form larger network structures in steps 2 and 3. The same holds for the differences between Lance and Warigal. Lance-lines are able to form larger particles than Warigal-lines. We believe this is due to differences in HMWGS composition.

However, the results with treatment #5 and $2+12$ lines are exceptional and do not fit the model. The $2+12$ lines behaved differently from the $5+10$ lines. In the heat
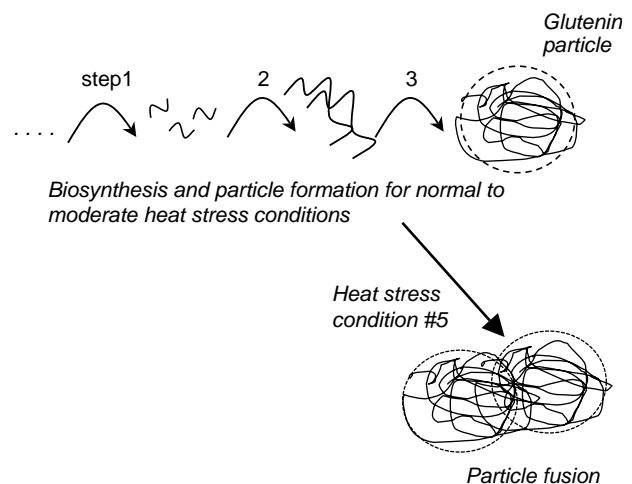

Fig. 10. Hypothetical formation of glutenin particles by hyper-aggregation and suspected particle fusion induced by heat stress condition #5.

treatment with $2+12$, more LMW subunits combined and may have had an unexpected effect on glutenin structure. D'Ovidio and Masci (2004) found that heat stress can affect the aggregation behaviour of LMW-GS. It is clear that the glutenin particle is different, from the results for particle structure, GMP rheology and CSLM. We propose that the large particles result from fusion between individual particles. Notably that this only occurs when heat stress is applied from 16 DAA. In condition #6, where stress is applied at 25 DAA, this phenomenon was not observed. In the greenhouse experiment with the Lance and Warigal lines, small quantities of 'polymeric' glutenin were observed about 16 DAA and possibly already at this time, these first formed particles could fuse under the high temperature conditions.

5. Conclusions

We have shown that heat stress can lead to a decrease in GMP with an increase of glutenin particles. In addition, we propose that severe heat stress conditions may even lead to fusion of glutenin particles. The increase in glutenin particle size will lead to an increase in DDT, whereas the decrease in GMP will lead to a lower dough stability (BWPR). Whether these typical effects on glutenin quantity, glutenin particle size and dough mixing are also reflected in bread-making quality was not assessed due to the small quantities of wheat grain material available.

Acknowledgements

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